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Validated RP-HPLC Method Development for the Simultaneous Estimation of Glycopyrrolate And Formoterol in its Combined Dosage Forms

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A B S T R A C T

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Glycopyrrolate and Formoterol in bulk and pharmaceutical formulations. Separation of Glycopyrrolate and Formoterol was successfully achieved on a Phenomenex Luna C18 (4.6×250mm, 5µm) particle size or equivalent in an isocratic mode utilizing Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) at a flow rate of 1.0mL/min and eluates was monitored at 245nm, with a retention time of 2.102 and 3.537 minutes for Glycopyrrolate and Formoterol respectively. The method was validated and the response was found to be linear in the drug concentration range of 10µg/mL to 50µg/mL for Glycopyrrolate and 20µg/mL to 100µg/mL for Formoterol. The values of the slope and the correlation coefficient were found to be 77824 and 0.999 for Glycopyrrolate and 10515 and 0.999 for Formoterol respectively. The LOD and LOQ for Glycopyrrolate were found to be 0.6µg/mL and 1.8µg/mL respectively. The LOD and LOQ for Formoterol were found to be 0.8 µg/mL and 2.4µg/mL respectively. This method was found to be good percentage recovery for Glycopyrrolate and Formoterol were found to be 100.351 and 100.93 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Glycopyrrolate and Formoterol, High performance liquid chromatography, Validation.

INTRODUCTION

Glycopyrrolate is a quaternary ammonium salt. Chemically, Glycopyrrolate is (RS)-[3(SR)- Hydroxy-1, 1-dimethylpyrrolidinium bromide] α-cyclopentylmandelate. The chemical formula is C19H28BrNO3 . The molecular weight is 398.33g/mol.1 Glycopyrrolate is a crystalline white powder. It is dissolvable in water and alcohol, and much insoluble in chloroform and ether[1]. Glycopyrrolate, as other anticholinergic (antimuscarinic) drugs, impedes the action of acetylcholine on structures innervated by postganglionic cholinergic nerves and on smooth muscles that respond to acetylcholine yet require cholinergic innervation. Thus, it diminishes the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal, and bronchial secretions[2].

Formoterol acts as a bronchodilator. It extends the airways of the lungs, so that it helps to inhale all the more effortlessly. It may even be utilized to forestall respiratory issues caused by exercise. It can also be utilized for long-term treatment of chronic obstructive pulmonary disease (COPD).4 Chemically, Formoterol is N-[2-Hydroxy5-[(1RS)-1-hydroxy-2-[[(1RS)-2(4-methoxyphenyl) -1-methylethyl]-amino] ethyl] phenyl] formamide (E)-2-butenedioate dihydrate[3]. The chemical formula is C19H24N2O4 . C4H4O4.2H2O. The molecular weight is 840.91g/mol.1 There are various analytical methods reported in the literature for the assay of Glycopyrrolate and Formoterol separately and also with other drugs include spectrophotometry, HPLC, HPTLC and other types. For only Glycopyrrolate separate RP HPLC methods were available in bulk, tablet dosage forms, and for parenterals. A survey of literature reveals that good analytical methods are not available for the drugs like Glycopyrrolate and Formoterol . Even though very few methods of estimation of above drugs are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity and simplicity etc[4].

The existing physicochemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for the assay of Glycopyrrolate and Formoterol in bulk form adapting available analytical techniques like HPLC. The present method was to build up a straightforward, minimal effort RP-HPLC technique for concurrent estimation of Glycopyrrolate and Formoterol in bulk and also in other dosage forms. The method was validated according to ICH guidelines[5].

MATERIALS AND METHODS

Materials

Glycopyrrolate and Formoterol were procured from Sura labs, Telangana. Water and Methanol for HPLC was procured from LICHROSOLV (MERCK). Acetonitrile for HPLC was purchased from Merck.

Instrumentation

Chromatographic conditions were developed for the analytical technique using Waters HPLC with auto sampler and PDA Detector 996 model. The column was Phenomenex Luna C18 with dimension 4.6mm×250mm length and particle size packing 5µm.

Preparation of mobile phase:

Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

System Suitability

Accurately weigh and transfer 10 mg of Glycopyrrolate and 10mg of Formoterol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.1ml of the above Glycopyrrolate and 0.3ml of the Formoterol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was determined.

Linearity

Accurately weigh and transfer 10 mg of Glycopyrrolate and 10mg of Formoterol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). Solutions were prepared containing 6ppm, 8ppm, 10ppm, 12ppm, 14ppm, concentrations of Glycopyrrolate and 18ppm, 24ppm, 30ppm, 36ppm, 42ppm, concentrations of Formoterol. Inject each level into the chromatographic system and measure the peak area.

Precision

Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of An analytical method is usually expressed as the standard deviation correlative standard deviation (coefficient of variation) of seriesof measurements

Accuracy

Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150 % of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For 50%, 150% concentration five sets and for 100% three sets were prepared and injected.

Robustness

The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 50:50, 40:60 instead (45:55), remaining conditions are same.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ was calculated from linear curve using formulae LOD=3.3*σ/slope, LOQ=10*σ/slope (Where σ=the standard deviation of the response and S= Slope of calibration curve).

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of Glycopyrrolate and Formoterol. The mobile phase containing Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) was found ideal to resolve the peak of Glycopyrrolate and Formoterol. Retention time of Glycopyrrolate and Formoterol

were 2.120 and 3.536 min respectively. System suitability parameters were evaluated and results shown in (Table-2), which were within acceptance criteria. Result of assay is shown in Table3. Results of intraday and interday precision were shown in the (Table-4&5). LOD and LOQ values were placed in Table-6. The robustness of the method was investigated by varying experimental conditions such as changes in flow rate and mobile phase. The result obtained implies method is robust for routine qualitative analysis (Table-7 &8).

Table 1 - Observations of sample Chromatogram.

S. No	Peak name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Glycopyrrolate	2.120	775684	13124		0.99	6365.0
2	Formoterol	3.536	2658478	937405	5.06	1.23	7458.0

Table 2-: Results of system suitability parameters for Glycopyrrolate and Formoterol

S.No	**Name**	**Retention time(min)**	**Area (µV sec)**	**Height (µV)**	**USP resolution**	**USP tailing**	**USP plate count**
1	Glycopyrrolate	2.120	765855.6	69558		1.6	5685
2	Formoterol	3.536	2535034	190085	2.01	1.6	5364.2

Table 3-: Results of Assay

S.No.	**Name of Compound**	**% Purity**
1	Glycopyrrolate	99.8%
2	Formoterol	99.6%

Table 4 -: Results of Intermediate precision for Formoterol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Formoterol	3.537	2653254	190110	5428	1.6
2	Formoterol	3.552	2648985	190058	5452	1.6
3	Formoterol	3.560	2658213	190142	5498	1.6
4	Formoterol	3.564	2653652	190031	5442	1.5
5	Formoterol	3.564	2648978	190058	5489	1.5
6	Formoterol	3.565	2658985	190047	5463	1.6
Mean			2653678			
Std. Dev			4313.355			
% RSD			0.162543			

Table 4a-: Results of Intermediate precision for Glycopyrrolate

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glycopyrrolate	2.102	766895	69858	5586	1.5
2	Glycopyrrolate	2.105	765988	69854	5636	1.6
3	Glycopyrrolate	2.112	766532	69824	5432	1.6
4	Glycopyrrolate	2.113	766214	69875	5468	1.6
5	Glycopyrrolate	2.109	765897	69854	5546	1.9
6	Glycopyrrolate	2.109	765245	69848	5507	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

Table 5-: Results of method precision for Formoterol

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Formoterol	3.552	2569865	2231111	5365	1.6
2	Formoterol	3.550	2578474	2674210	5425	1.6
3	Formoterol	3.564	2568985	2231261	5368	1.5
4	Formoterol	3.564	2586845	2421301	5359	1.5
5	Formoterol	3.565	2545898	2324710	5498	1.6
Mean			2570013			
Std. Dev			15309.45			
% RSD			0.595695			

Table 5a-: Results of method precision for Glycopyrrolate

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Glycopyrrolate	2.108	766854	702564	5685	1.6
2	Glycopyrrolate	2.105	765884	698789	5584	1.4
3	Glycopyrrolate	2.113	765842	701235	5521	1.6
4	Glycopyrrolate	2.109	768985	700124	5525	1.9
5	Glycopyrrolate	2.109	765845	698986	5578	1.7
Mean			766682			
Std. Dev			1357.973			
% RSD			0.177123			

Table 6: LOD and LOQ

S.No.	**Name of Compound**	**LOD (µg/ml)**	**LOQ (µg/ml)**
1	Glycopyrrolate	0.6	1.8
2	Formoterol	0.8	2.4

Table 7-: Robustness – variation in flow-System suitability results for Escitalopram

		System Suitability Results	
S.No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.9	5784.6	1.06
2	1.0	5685.4	1.08
3	1.1	5869.5	1.09

System suitability results for Clonazepam

		System Suitability Results	
S.No	**Flow Rate (ml/min)**	**USP Plate Count**	**USP Tailing**
1	0.9	6698.3	1.46
2	1.0	6895.7	1.42
3	1.1	6983.6	1.49

Table 8-: Robustness – variation in mobile phase-System suitability results for Glycopyrrolate

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765789	2.102	5587	1.7
Less Flow rate of 0.9 mL/min	758698	2.330	5458	1.7
More Flow rate of 1.1 mL/min	7689584	1.950	5696	1.7
Less organic phase	758412	2.290	5586	1.4
More organic phase	769852	1.998	5355	1.5

Robustness – variation in mobile phase-System suitability results for Formoterol

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532158	3.537	5398	1.6
Less Flow rate of 0.9 mL/min	2458692	3.885	5329	1.7
More Flow rate of 1.1 mL/min	2658642	3.263	5256	1.7
Less organic phase	2452148	4.435	5214	1.2
More organic phase	2653894	3.009	5524	1.0

DISCUSSION and CONCLUSION

A new method was established for simultaneous estimation of Glycopyrrolate and Formoterol by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Glycopyrrolate and Formoterol by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid), detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Glycopyrrolate and Formoterol was found to be 99.8%. The system suitability parameters for Glycopyrrolate and Formoterol such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Glycopyrrolate and Formoterol was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Glycopyrrolate and Formoterol in API and Pharmaceutical dosage form. The proposed RP-HPLC method was used for the simultaneous estimation of Glycopyrrolate and Formoterol was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing Glycopyrrolate and Formoterol.

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